13. (Amended) The method of claim 12 comprising the steps of detecting the presence of an insert having SEQ ID NO:26.

15. (Amended) The method of claim 12, wherein the sasp-B gene primers hybridize to the forward and reverse strands of sequence of SEQ ID NO:11.

In the Drawings:

Please amend Figures 1C, 2A, 2C and 3 as follows (see attached sheets):

REMARKS

Claims 1-18 are pending in this application. Claims 2, 4, 5, 7, 8, 11, 13 and 15 have been amended. The amendments to claims 2, 4, 5, 7, 8, 11, 13 and 15 correct or insert the assigned identifier (SEQ ID NO:) for sequences designated in these claims.

The amendment to paragraph **[58]** beginning on page 19, line 14, correct errors of a typographical nature made without deceptive intent. This amendment inserts missing bases at their proper locations to bring the "Bcer" and "Banth" sequences into alignment. Justification for these corrections may be found at page 9, lines 29-37 and page 10, lines 1-3; page 17, lines 33-34 and page 18, lines 1-12; and Fig. 1A-1C and Fig. 2A-2C, where the correct sequences for SEQ ID NOS:10 and 11 (identifiers amended) occur. This amendment also corrects the location of dashed lines and arrows under the sequences to properly indicate nucleotide positions corresponding to the oligonucleotide probes BaSPB2, BaSPB4 and BASPB5, given on page 20, lines 4-6, as SEQ ID NOS:34-36 (identifiers amended).

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above

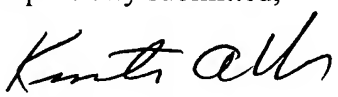
named sequences, SEQ ID NOS:1-38, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification and Claims by the current Amendment. The attached pages are captioned **"VERSION WITH MARKINGS TO SHOW CHANGES MADE."** As a convenience to the Examiner, a complete set of the Claims, as amended herein, is also attached to this Amendment as an Appendix entitled **"PENDING CLAIMS WITH ENTRY OF THE AMENDMENT."**

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph **[21]** beginning at line 2 of page 5 has been amended as follows:

[21] Figure 1. is a multiple ClustalW DNA Sequence Alignment of sasp-B Amplicons from 38 *Bacillus anthracis* strains (SEQ ID NO:11) ~~(Seq. ID No. 13 through 50)~~. Bases 1 - 90 are in Fig. 1A, 91 - 180 are in Fig. 1B ~~1-B~~, and 181 - 240 are in Fig. 1C.

Paragraph **[22]** beginning at line 5 of page 5 has been amended as follows:

[22] Figure 2. is a ClustalW multiple sasp-B DNA Sequence Alignment of *Bacillus anthracis*, *Bacillus thuringiensis* and *Bacillus cereus* strains (SEQ ID NOS:12-15, 10, 16, 17-21 and 11, respectively) ~~(Seq. ID No. 51 through 87)~~. Bases 1 - 90 are in Fig. 2A, 91 - 180 are in Fig. 2B ~~2-B~~, and 181 - 240 are in Fig. 2C.

Paragraph **[23]** beginning at line 9 of page 5 has been amended as follows:

[23] Figure 3. is a representation of *Bacillus globigii* specific PCR primers targeting Bg sasp-gamma (SEQ ID NOS:24 and 25). *B. globigii* sasp-gamma (BgSSPE_edited_) = SEQ ID NO:22; *B. subtilis* sasp-gamma (Bs_pub_SSPE) = SEQ ID NO:23).

Paragraph **[26]** beginning at line 4 of page 6 has been amended as follows:

[26] Primer sequences were located within each sasp sequence which would maximize the likelihood of amplifying non-homologous sequences. For instance, whenever possible the 3' end of a primer was concluded with one or more thymidine residues. Potential primer sequences were analyzed using Oligo 4.0 primer design software (National Biosciences, Plymouth, MN) for potential hairpin or concatomers, which might interfere with hybridization to target DNA. Also using Oligo 4.0 primer design software (National Biosciences, Plymouth, MN), primer sequences were adjusted to match their melting temperatures as closely as possible to one another, which generally enhances reaction specificity. The sequence similarity search tool BLAST was queried with the primer sequences in order to insure that the primers did not recognize any bacterial (or other microbial) sequences except the targeted *Bacillus* species. Primers were synthesized (SEQ ID NOS:1-6) ~~(Sequence IDs No.1 through 6)~~ using the PerSeptive Biosystems Expedite nucleic acid synthesis system (Perkin Elmer, Norwalk, Conn.). Oligos were released from columns by incubation in 29.3% ammonium hydroxide at 55°C. overnight, followed by evaporation of ammonium hydroxide using the SpeedVac 1SS110 (Savant Corp.). Primers were resuspended in 10 millimolar tris buffer, pH 8.3, and their concentration measured with a spectrophotometer.

Paragraph **[36]** beginning at line 29 of page 8 has been amended as follows:

[36] In these alignments, dots signify a match with the sequence shown; only mismatches are spelled out, in order to emphasize them. Primer sequences are not included, but would be extensions of the 5' and 3' ends of the sequences shown.

The *Bacillus anthracis* and *B. cereus* sasp-1 sequence alignment did not show significant differences

```
B.cer     1 CGTAATGAAGTATTAGTTCGAGGCGCTGAACAAGCTCTTGATCAAATGAAATATGAAATT
B.anth    1 .........T............T.............................

B.cer    61 GCACAAGAGTTTGGTGTACAACTTGGTGCAGATACAACAGCTCGTTCAAACGGATCTGTT
B.anth   61 ................................................T.........

B.cer   121 GGTGGTGAAATTACAAAACGTTTAGTAGCAATGGCAGAACA (SEQ ID NO: 7)
B.anth  121 ................................T..... (SEQ ID NO: 8)
```

The *Bacillus anthracis* and *B. cereus* sasp-2 sequence alignment did not show significant differences

```
B.cer     1 AGCGGTTCCTGGTGCTGAATCAGCATTAGACCAAATGAAATACGAAATCGCTCAAGAGTT
B.anth    1 ............................................................

B.cer    61 TGGTGTTCAACTTGGAGCTGATGCAACAGCTCGCGCTAACGGTTCTGTTGGTGGCGAAAT
B.anth   61 ............................................................

B.cer   121 CACTAAACGTCTAGTTTCACTAGCTGAGCAACAA (SEQ ID NO: 9)
B.anth  121 ..............................  (SEQ ID NO: 9)  (SEQ ID
NO: 10)
```

The *Bacillus anthracis* and *B. cereus* sasp-B sequence alignment showed a significant difference, namely a TAGCATT (SEQ ID NO:26) insert

```
BcerPub    1 AACAAAGCAACTTCTGGTGCTAGCATTCAAAGTACAAATGCTAGTTATGGTACAGAGTTT
Banth      1 .....G.....................C.....................

BcerPub   61 TCAACTGAAACAGATGTACAAGCTGTAAAACAAGCAAACGCACAATCAGAAGCAAAGAAA
Banth     61 G.G.........A..........A.............................T......

BcerPub  121 GCACAAGCTTCTGGTGCA------CAAAGTGCAAACGCTAGTTATGGTACAGAATTTGCA
Banth    121 ..G.............TAGCATT.....CA....T.....................

BcerPub  175 ACTGAAACAGACGTGCATTCTGTGAAAAAACAAAATGCTAAGTCAGCTGCAAAACAA
               (SEQ ID NO:10) (SEQ ID NO: 11)
Banth    181 .................G.................AC.A.............
               (SEQ ID NO:11) (SEQ ID NO: 12)
```

Paragraph **[38]** beginning at line 9 of page 10 has been amended as follows:

[38] The underlined sequence TAGCATT (SEQ ID NO:26) ~~(SEQ ID NO 107)~~ represents an insertion region useful for distinguishing *Bacillus anthracis* from other *Bacillus* species.

Paragraph **[45]** and TABLE 2. beginning at line 3 of page 13 has been amended as follows:

[45] Referring now to Figs. 2A, 2B, and 2C, the single *Bacillus anthracis* sequence (#37 which is the bottom row of Figs. 2A, 2B, & 2C) shows a unique pattern of sequence divergence from the sasp-B sequence of these near neighbor isolates. The identities of the sequences are shown in Table 2 below:

TABLE 2.

Legend:

Bacteria	BGSC#	Serotype		Designation
Bacillus licheniformis,		5A2		5A2
Bacillus thuringiensis		4A1	serot-1	4A1
Bacillus thuringiensis		4A3	cry (thur) serot-1	4A3
Bacillus thuringiensis		4J2	aizawai, pacificus/serot-7	4J2
Bacillus thuringiensis	HD3	4B2	2 standard	BtB
Bacillus thuringiensis	HD4	4C3	3a standard	BtC
Bacillus thuringiensis	HD7	4E2	4a4b dendrolimus standard	BtE2
Bacillus thuringiensis		4E4	4a4b	BtE4
Bacillus thuringiensis	HD29	4G5	5a5b	BtG
Bacillus thuringiensis	HD10	4I1	6	BtI
Bacillus thuringiensis	HD11	4J4	7	BtJ
Bacillus thuringiensis	HD12	4K1	8 standard	BtK
Bacillus thuringiensis	HD537 4L3	9 standard		BtL
Bacillus thuringiensis	HD146 4M1	10 standard		BtM
Bacillus thuringiensis	HD201 4N1	11 antisera standard		BtN ~~BtM~~
Bacillus thuringiensis	HD542 4O1	12 standard		BtO
Bacillus thuringiensis	HD395	4P1	13 standard	BtP
Bacillus thuringiensis	ONR60A	4Q1	14	BtQ
Bacillus thuringiensis	HD511 4R1	15		BtR
Bacillus thuringiensis	HD521 4S2	16 standard		BtS
Bacillus thuringiensis	HD525 4T1	no flagellar antigen		BtT
Bacillus thuringiensis	HD541 4U1	11a11c		BtU
Bacillus thuringiensis		4V1	17	BtV
Bacillus thuringiensis	HD 867	4W1	18	BtW
Bacillus thuringiensis	IS720	4X1	21	BtX
Bacillus thuringiensis	HD868	4Y1	19 standard	BtY
Bacillus thuringiensis	HD501	4Z1	8a8c standard	BtZ
Bacillus anthracis	BA42D	11		NMRI#11

Unidentified *Bacillus*			003
Unidentified *Bacillus*		Taken from filled bag in "final mixing trailer"	1B
Unidentified *Bacillus*		Isolated from 1B culture as morphologically distinct colonies	1B/A
Unidentified *Bacillus*		Isolated from 25kg media drum, bentonite mixture	III
Unidentified *Bacillus*		Isolated from bentonite spore stock	IV
Bacillus cereus	Genbank #M16813	NCBI Genbank database	Bcerpub
Bacillus cereus	ATCC 14579	Purchased from ATCC	Bcer1
Bacillus cereus	ATCC 11778	Purchased from ATCC	Bcer2
Bacillus cereus	ATCC 6464	Purchased from ATCC	Bcer3

BGSC is the Bacillus Genetic Stock Center, at The Ohio State University

Paragraph **[46]** and TABLE 3. beginning at line 4 of page 15 has been amended as follows:

[46] Based on the DNA sequence information in Figures 1 and 2, amino acid sequences were extrapolated and evaluated for the sasp-B genes from *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis* ~~Bacillus thuringiensis~~. These extrapolated sequences are shown below in an extrapolated amino acid sequence alignments for the sasp-B gene from *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis* Bacillus thuringiensis. The identities of the sequences are shown in Table 3.

```
          1              15 16             30 31             45 46             60
1 4D4     NKATSGASIQSTNAS YGTEFSTETDVQAVK QANAQSEAKKAQASG A-QSANASYGTEFA
2 Bcerp Bcep   NKATSGASIQSTNAS YGTEFSTETDVQAVK QANAQSEAKKAQASG A--
QSANASYGTEFA
3 BtK     NKATSGASIQSTNAS YGTEFATETNVQAVK QANAQSEAKKAQASG A--QSANASYGTEFA
4 BtB     NKATSGASIQSTNAS YGTEFSTETDVQAVK QANAQSEAKKAQASG A--QSANASYGTEFA
5 Banth   NKATSGASIQSTNAS YGTEFATETNVQAVK QANAQSEAKKAQASG ASIQSTNASYGTEFA
6 Bmyc    NKATSGASIQSTNAS YGTEFATETNVQAVK QANAQSEAQKAQASA A--QSANASYGTEFA

          61             75 76
1 4D4     TETDVHSVKKQNAKS AAKQ (SEQ ID NO:27) (SEQ ID NO: 88)
2 Bcerp Bcep   TETDVHSVKKQNAKS AAKQ (SEQ ID NO:27) (SEQ ID NO: 89)
3 BtK     TETDVHAVKKQNAKS AAKQ (SEQ ID NO:28) (SEQ ID NO: 90)
4 BtB     TETDVHAVKKQNAQS AAKQ (SEQ ID NO:29) (SEQ ID NO: 91)
5 Banth   TETDVHAVKKQNAQS AAKQ (SEQ ID NO:30) (SEQ ID NO: 92)
6 Bmyc    TETDVHAVKKQNAQS AAK  (SEQ ID NO:31) (SEQ ID NO: 93)
```

TABLE 3.

Legend:

Bacteria	BGSC#	Serotype	Designation
Bacillus thuringiensis type strain			4D4
Bacillus thuringiensis	HD12	4K1 8 standard	BtK
Bacillus thuringiensis	HD3	4B2 2 standard	BtB
Bacillus cereus published sequence		GenBank #M16813	Bcerp
Bacillus mycoides		ATCC 6421, subtype Flugge	Bmyc
~~*Bacillus mycoides*~~		~~ATCC 6421, subtype Flugge~~	~~Bmyc~~
Bacillus anthracis			Banth

Paragraph **[47]** beginning at line 7 of page 16 has been amended as follows:

[47] In the previous examples, the BcSasp-B primers were useful for evaluating the prevalence of the unique *Bacillus anthracis* sasp-B signature, but sequencing was required to distinguish amplicons of the several *Bacillus* ~~Bacillus~~ species which could be amplified using the *Bacillus cereus* primers. By studying the alignment of *Bacillus anthracis* and *Bacillus cereus* sasp-B sequences (above) potential anthracis specific primer and probe sites were identified (shown below, SEQ ID NOS:32 and 33

~~SEQ ID NO: 94 and 95~~). Eight oligonucleotides were designed with the aid of Oligo 4.0 and BLAST database search utilities then synthesized (all as described in Example 1 above) and evaluated experimentally in various combinations for their ability to prime amplification of *Bacillus anthracis* only, using a panel of near neighbor *Bacillus* ~~Bacillus~~ species. Three of the primer pairs were designed to incorporate the *Bacillus anthracis* insertion region into the three prime end of one primer per pair. This strategy greatly limited amplicon size and did not leave any *Bacillus anthracis* specific sequence for probe design.

Paragraph **[48]** beginning at line 19 of page 16 has been amended as follows:

[48] The combination of primers originally designated BaSPB7 and BaSPB8 (below) were sufficiently specific. From 100 nanograms *B. cereus* target a very faint product band of nearly (but not exactly) the correct size, was evident; when compared to signals from an amplified dilution series of *Bacillus anthracis* DNA, the signal from *Bacillus cereus* was approximately equivalent to product from 10 picograms - indicating 10,000 fold less efficient amplification. Bands were not visible at or near the correct size from products of *Bacillus coagulans* ~~Bacillus coagulans~~, *Bacillus circulans* ~~Bacillus circulans~~, *Bacillus globigii*, *Bacillus mycoides* ~~Bacillus mycoides~~, *Bacillus subtilis* ~~Bacillus subtilis~~ or *Bacillus thuringiensis* ~~Bacillus thuringiensis~~ amplification.

Paragraph **[49]** beginning at line 27 of page 16 has been amended as follows:

[49] In addition, these primers were for sequences flanking, rather than incorporating the *Bacillus anthracis* insertion region, thus leaving this region within the product for binding to probes designed to hybridize to this unique signature.

The *Bacillus anthracis* primer data (from analysis by Oligoprimer design software, National Biosciences, Plymouth, MN.) is summarized as follows:

BaSPB7 primer sequence:

5' GTT ATG GTA CAG AGT TTG CG 3' (SEQ ID NO:32) ~~(SEQ ID NO: 94)~~

Tm = 57.4 °C (salt 1000.0 mM; oligo 0.6 pM)

Td = 57.6 °C, G(25°C) = -34.7 kcal/mol, Mr = 6283

Ext. coeff.: 5.05 nmol/A260, 31.7 µg/A260

BaSPB8 primer sequence:

5' TTG TTT TGC AGC TGA TTG T 3' (SEQ ID NO:33) ~~(SEQ ID NO: 95)~~

Tm = 58.3 °C (salt 1000.0 mM; oligo 0.6 pM)

Td = 58.9 °C, G(25°C) = -34.1 kcal/mol, Mr = 5911

Ext. coeff.: 5.82 nmol/A260, 34.4 µg/A260

Paragraph **[51]** beginning at line 20 of page 17 has been amended as follows:

[51] Thermalcycling: Amplifications were performed in a Perkin Elmer 9600 thermocycler with the following thermal cycling regime: 94°C ~~94oC~~ for 5 minutes, then 40 repeating cycles of 94°C ~~94oC~~ for 30 seconds, 50°C ~~50oC~~ for 30 seconds and 72°C ~~72oC~~ for 30 seconds, followed by a 7 minute 72°C ~~72oC~~ final extension step.

Paragraph **[52]** beginning at line 24 of page 17 has been amended as follows:

[52] Reaction mixture: Each 100ul reaction contained 0.1 millimolar each dATP, dCTP, dGTP and dTTP, 25 picomoles each primer, 10 millimolar Tris-HCl pH 8.3, 2 millimolar $MgCl_2$ ~~MgCl2~~, 25 millimolar KCl, 2.5 units of Taq polymerase (Perkin Elmers, Norwalk, Conn.) and 100 ng or less of template DNA.

The uniqueness of these primers may be seen by a *Bacillus anthracis* and *Bacillus cereus* sasp-B sequence alignment emphasizing *Bacillus anthracis* specific primer sequences:

```
BcerPub    1 AACAAAGCAACTTCTGGTGCTAGCATTCAAAGTACAAATGCTAGTTATGGTACAGAGTTT
Banth      1 .....G.....................C.........GTTATGGTACAGAGTTT
                                                   --primer BaSPB7--

BcerPub   61 TCAACTGAAACAGATGTACAAGCTGTAAAACAAGCAAACGCACAATCAGAAGCAAAGAAA
Banth     61 GCG.........A.........A.............................T......
               -->  (SEQ ID NO:32)

BcerPub  121 GCACAAGCTTCTGGTGCA------CAAAGTGCAAACGCTAGTTATGGTACAGAATTTGCA
Banth    121 ..G.............TAGCATT.....CA....T.....................

BcerPub  175 ACTGAAACAGACGTGCATTCTGTGAAAAAACAAAATGCTAAGTCAGCTGCAAAACAAA
(SEQ ID NO:10) (SEQ ID NO: 96)
Banth    181 .................G.................ACAATCAGCTGCAAAACAAA
(SEQ ID NO:11) (SEQ ID NO: 97)
                                    (SEQ ID NO:33) <--primer BaSPB8-----
```

Paragraph **[53]** beginning at line 15 of page 18 has been amended as follows:

[53] The *Bacillus anthracis* and *Bacillus cereus* ~~Bacillus ceurus~~ sasp-B sequence alignment shows the sequence similarity between the present *Bacillus anthracis* sasp B DNA (which is a 240 base pair amplicon as described above) and the corresponding region of its most similar known sequence, the *Bacillus cereus* ~~Bacillus ceurus~~ sasp B gene. This alignment, run in the CLUSTALW program described herein, yields a similarity score of 89%, using default parameters. As is known in the art, the default parameters for nucleic acid pairwise alignments are gap opening penalty = 15; gap extension penalty = 6.66. The IUB matching protocol is used -All matches score 1.9; all mismatches for IUB symbols score 0. The CLUSTAL matching protocol can also be

used. In this case, matches score 1.0 and mismatches score 0.0. In either case, CLUSTALW comparison of the *Bacillus anthracis* sasp B DNA and the comparable *Bacillus cereus* ~~Bacillus ceurus~~ sasp B DNA yields a score of 89%.

Paragraph **[55]** beginning at line 31 of page 18 has been amended as follows:

[55] In the present case, a BLAST search revealed that the closest sequence corresponds to the *Bacillus cereus* ~~Bacillus ceurus~~ small acid-soluble spore protein, Accession Number M16813. This corresponds with the discussion in this Example. Using BLAST parameters, the two nucleotide sequences have an identity of 86%.

Paragraph **[57]** beginning at line 6 of page 19 has been amended as follows:

[57] Accordingly, those of skill in the art would recognize that the Sasp-B DNA of *Bacillus anthracis* is homologous but not identical to that of *Bacillus cereus*. Thus, this invention includes any sasp-B from *Bacillus anthracis* that might include minor single base differences (polymorphisms) from SEQ ID NO:11 ~~SEQ ID NO: 97 (or identical SEQ ID NO:87)~~, yet, maintain the insert of SEQ ID NO:26 ~~SEQ ID NO: 107~~.

Paragraph **[58]** beginning at line 14 of page 19 has been amended as follows:

[53] Three of the oligonucleotides evaluated as primers incorporated the *Bacillus anthracis* specific insertion region, and having designed primers flanking the insertion region, these oligos were tested as probes to confirm the identity of the amplicons; only amplicons from *B. anthracis* would include the 6 base insertion, as follows:

Alignment of *Bacillus cereus* and *Bacillus anthracis* Sasp-B sequences emphasizing probed locations

```
Bcer   AACAAAGCAACTTCTGGTGCTAGCATTCAAAGTACAAATGC
Banth  AACAAGGCAACTTCTGGTGCTAGCATTCAAAGCACAAATGC

Bcer   TAGTTATGGTACAGAGTTTTCAACTGAAACAGATGTACAAGCTGTAAAACAAGCAAACGCACAA
Banth  TAGTTATGGTACAGAGTTTGCGACTGAAACAAATGTACAAGCAGTAAAACAAGCAAACGCACAAT

Bcer   TCAGAAGCAAAGAAAGCACAAGCTTCTGGTGCA------CAAAGTGCAAACGCTAGTTATGGTACAGAATTTGCAA
Banth  TCAGAAGCTAAGAAAGCGCAAGCTTCTGGTGCTAGCATTCAAAGCACAAATGCTTTGCATAGTTATGGTACAGAAA
                                  ---------- BaSPB4
                                  ----------
                                  ---------- BaSPB2
                                  ----------
                                  ↑  location of probes tested  ↑
                                  ↑ location of probes tested ↑
```

```
Bcer   CTGAAACAGACGTGCATTCTGTGAAAAAACAAAATGCTAAGTCAGCTGCAAAACAA (SEQ ID NO:10) (SEQ ID NO: 98)
Banth  CTGAAACAGACGTGCATGCTGTGAAAAAACAAAATGCACAATCAGCTGCAAAACAA (SEQ ID NO:11) (SEQ ID NO: 99)
```

Three oligonucleotides evaluated for use as *Bacillus anthracis* probes:

BaSPB2: (inverted, 'lower strand' sequence): 5' GCATTTGTGCTTTGAATGCTA 3' (SEQ ID NO:34) (SEQ ID NO: 100)

BaSPB4: (inverted, 'lower strand' sequence): 5' CATTTGTGCTTTGAATGCTA 3' (SEQ ID NO:35) (SEQ ID NO: 101)

BaSPB5: (direct, 'upper strand' sequence): 5' AGCTTCTGGTGCTAGCATT 3' (SEQ ID NO:36) (SEQ ID NO: 102)

Paragraph **[63]** beginning at line 34 of page 20 has been amended as follows:

[63] Finally, probe BaSPB4 was tested for specificity. Fifty nanograms of DNA from each of the following *Bacillus* ~~Bacillus~~ species was amplified using biotinylated BaSPB7 and BaSPB8: *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus*

cereus, *Bacillus mycoides* ~~Bacillus mycoides~~, *Bacillus subtilis* ~~B. subtilis~~, and *Bacillus globigii*. The denatured amplicon of each DNA species was reacted against the bound BaSPB4 probe and the test strips developed. Only the *Bacillus anthracis* amplicon resulted in any signal (which was quite intense); none of the other species bound to the probe in order to result in a signal. Conclusion: BaSPB4 binds *Bacillus anthracis* DNA specifically. BaSPB4 binding specificity was demonstrated with the BioRad universal GeneComb System (data not shown). 100 ng of each species of DNA amplified was placed on each panel, as follows (1 - 8):

Species Amplified:

1) *Bacillus anthracis*
2) *Bacillus thuringiensis*
3) *Bacillus cereus*
4) *Bacillus mycoides* ~~Bacillus mycoides~~
5) *Bacillus subtilis* ~~Bacillus subtilis~~
6) *Bacillus globigii*
7) Negative PCR
8) Kit positive control

Paragraph **[71]** beginning at line 16 of page 22 has been amended as follows:

[71] In the same manner as described in Example 1 above, a sasp gene (sasp-gamma in this case) sequence was identified for the production of primers specific for *Bacillus globigii* sequence. Primers and amplification conditions were designed (see Figure 3) for heterologous PCR based on published sequence for the *Bacillus subtilis* ~~Bacillus subtilis~~ sasp E gene (sasp-gamma) acquired from GenBank (accession number M16184). After sequencing amplicons from *Bacillus globigii* (generated using the *Bacillus subtilis* ~~Bacillus subtilis~~ primers), and aligning *Bacillus globigii* sequence with the published *Bacillus subtilis* ~~Bacillus subtilis~~ sequence, *Bacillus globigii* specific

primers were designed taking advantage of the differences in the sequence. After searching the databases to be sure that the new *Bacillus globigii* primers were not homologous to other sequences, and optimizing amplification conditions, a panel of *Bacillus* ~~Bacillus~~ species were amplified to check primer specificity. Amplicons of the correct size were produced only from *Bacillus* ~~Bacillus~~ designated as *Bacillus globigii*, for all but the most arcane intents and purposes (there is disagreement among a very few researchers ~~researcher~~ as to whether *Bacillus subtilis niger* ~~Bacillus subtilis niger~~ and *Bacillus atrophaeus* ~~Bacillus atrophaeus~~ are, in fact, genetically different from *Bacillus globigii* at all); importantly, the new primers did not amplify *Bacillus subtilis* ~~Bacillus subtilis~~ or *Bacillus amyloliquifaciens* ~~Bacillus amyloliquifaciens~~ - which are distinct species, yet very closely related to *Bacillus globigii* ~~Bacillus globigii~~. Referring now to Fig. 3, there is shown an alignment of *Bacillus subtilis* ~~Bacillus subtilis~~ sasp-gamma sequence (from Genbank) (Bs_pub_SSPE) with *Bacillus globigii* sequence (upper strand) showing the location of the primer sequences and how their sequence compares to the known *Bacillus subtilis* ~~Bacillus subtilis~~ sequence.

Paragraph **[72]** beginning at line 3 of page 23 has been amended as follows:

[72] The BgSaspGam primers produce *Bacillus globigii* ~~B globigii~~ specific PCR product, as was demonstrated in an Nuseive-Agarose gel (data not shown). The gel showed approximately a 135b *Bacillus globigii* ~~Bacillus globigii~~ specific amplicon. No amplification of negative controls in *Bacillus cereus*; *Bacillus amyoliquifaciens* ~~Bacillus amyoliquifaciens~~, *Bacillus megaterium* ~~Bacillus megaterium~~, or *Bacillus globisporus* ~~Bacillus globisporus~~ was observed. Amplification was observed with *Bacillus atropheus* ~~Bacillus atropheus~~ (ATCC 6455 and 49337) and *Bacillus subtilis niger* ~~Bacillus niger~~. It should be noted that *Bacillus subtilis niger* ~~Bacillus subtilis niger~~ and *Bacillus atrophaeus* ~~Bacillus atrophaeus~~ have been officially designated *Bacillus globigii* since they are virtually indistinguishable from *Bacillus globigii* at the molecular level. Near neighbors *Bacillus subtilis* ~~Bacillus subtilis~~, *Bacillus globisporus* ~~Bacillus globisporous~~

and *Bacillus megatarium* ~~Bacillus megatarium~~ do not amplify with the BgSaspGam primers.

Bacillus globigii sasp-gamma primers:

BgSaspGam 5' 5' ACATGGCTAACTCAAACAACAA 3' (SEQ ID NO:24) ~~(SEQ ID NO: 103)~~

BgSaspGam 3' 5' GGTTTTGTTTTCTTACTTGTTGTAC 3' (SEQ ID NO:25) ~~(SEQ ID NO: 104)~~

Paragraph **[77]** beginning at line 2 of page 24 has been amended as follows:

[77] In a manner similar to the above descriptions, a sasp gene (sasp-2 in this case) sequence was identified for the production of primers for amplification of *Clostridium perfringens* sequence. Primers and amplification conditions were designed and carried out using *Clostridium perfringens* DNA. While amplification successfully produced product of the correct size (when viewed by ethidium bromide gel electrophoresis), near neighbor DNA has yet to be evaluated in order to assess specificity of these primers.

Clostridium perfringens sasp-2 primers:

CPssp2-1: 5' -AATAACTAAGGAGGAATGAAAAATGT— 3' (SEQ ID NO:37) ~~(SEQ ID NO:105)~~

Cpssp2-2: 5' -TTGTTCTACCATTCTTTTAACCATT —3' (SEQ ID NO:38) ~~(SEQ ID NO: 106)~~

Paragraph **[83]** beginning at line 35 of page 24 has been amended as follows:

[83] Having described the present invention, it will be apparent that other embodiments are possible in light of the present teachings. For example, other DNA amplification methods besides PCR are known, such as the Q-beta replicase method. Certain of these methods may be used in a single-step amplification/detection protocol, based, for example, on the unique *Bacillus anthracis* sasp-B insertion TAGCATT (SEQ ID NO:26) ~~(SEQ ID NO 107)~~.

Paragraph **[85]** beginning at line 10 of page 25 has been amended as follows:

[85] The novel sasp polypeptides of the invention can also be used to produce antibodies which are specifically immunoreactive or bind to epitopes of the sasp polypeptides. Antibodies of the invention specifically include antibodies which bind to unique polypeptides produced by the *B. anthracis* DNA sequence shown in Example 5 ~~Example 6~~ (and translated in Example 6 ~~Example #5~~) and identified as NMRI#11.

In the Claims:

Claims 2, 4, 5, 7, 8, 11, 13 and 15 have been amended as follows:

2. (Amended) The nucleic acid of claim 1 having the nucleic acid sequence of SEQ ID NO:11 ~~SEQ ID NO: 87~~.

4. (Amended) The nucleic acid of claim 1 having the nucleic acid sequence of SEQ ID NO:26 ~~SEQ ID NO: 107~~.

5. (Amended) The nucleic acid of claim 1, wherein the nucleic acid encodes SEQ ID NO:30 ~~SEQ ID NO: 92~~.

7. (Amended) The antibody of claim 6, wherein the *Bacillus anthracis* sasp-B protein has an amino acid sequence of SEQ ID NO:30 ~~SEQ ID NO: 92~~.

8. (Amended) The antibody of claim 6, wherein the antibody binds to the epitope encoded by TAGCATT (SEQ ID NO:26).

11. (Amended) A nucleic acid probe that hybridizes to the sequence 5'-TAGCATT-3' (SEQ ID NO:26) ~~5' TAG CAT T 3"~~ or the complimentary strand thereof.

13. (Amended) The method of claim 12 comprising the steps of detecting the presence of an insert having SEQ ID NO:26 ~~SEQ ID NO: 107~~.

15. (Amended) The method of claim 12, wherein the sasp-B gene primers hybridize to the forward and reverse strands of sequence of SEQ ID NO:11 ~~SEQ ID NO: 87~~.

In the Drawings:

Figures 1C, 2A, 2C and 3 have been amended as follows (see attached sheets):